The essence of cytokines from experimental acute pancreatitis through organ preserving pancreatic head resection

Ph. D. THESIS

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1. INTRODUCTION

Acute pancreatitis

Acute pancreatitis is a relatively common disease with an annual incidence of 10-20 cases per 100,000, population in the Western world. The majority of cases are alcohol related or due to gallstones. Although many etiological factors are known to be involved in triggering acute pancreatitis, however, once the inflammatory process has been initiated, the ultimate outcome is relatively independent of the causative agent. Furthermore, acute pancreatitis has a wide spectrum of clinical manifestations, which ranges from a mild oedematous, self-limited disease with a fair prognosis to severe necrotizing inflammation with a fatal outcome. Despite numerous experimental and clinical results, the real pathomechanism of acute and chronic pancreatitis has not been established in detail yet. Over the last 15 years the understanding and management of acute pancreatitis has radically altered.

The initial events in acute pancreatitis might occur within the acinar cells Zymogen and lysosomal granules are made fragile by substances such as alcohol and its metabolites. In gallstone pancreatitis, these granules appear colocalized within acinar cells. Products released by the granules lead to intracellular activation of digestive enzymes, such as trypsin, and acinar cell injury. The initial response is closely followed by a second stage consisting of immigration of leukocytes into the pancreas, due to inflammatory mechanisms. This important phenomenon is driven by cytokines, chemokines and other inflammatory mediators secreted by resident cells belonging to the innate immune system and parenchymal and mesenchymal cells

1.2. Chronic pancreatitis

In summary, CP is characterized by progressive and ultimately irreversible pancreatic injury that manifests clinically as maldigestion and diabetes. Alcohol abuse is the most common association of CP in the Western world. Important advances have been made in recent years with respect to our understanding of the pathogenesis of this disease, particularly related to the mechanisms responsible for the development of pancreatic fibrosis (a cardinal feature of CP) after repeated acute attacks of pancreatic necroinflammation (the necrosis-fibrosis concept). The pancreatic stellate cell is now established as playing a central role in fibrogenesis, particularly when activated either directly by toxic factors associated with pancreatitis (such as ethanol, its metabolites, or oxidant stress) or by cytokines released during pancreatic necroinflammation.

1.3. Cytokines

Cytokine is a term applied to any of a rapidly growing number of small, nonstructural proteins or glycoproteins that serve as messengers between cells and are involved in such processes as cell growth and differentiation, tissue repair and remodeling, and regulation of the immune response. In acute and chronic inflammation, cytokines are instrumental in regulating the magnitude, nature, and duration of the inflammatory response. Cytokines also stimulate or inhibit the development of hematopietic cells.

General properties of cytokines: they are polypeptides produced in response to microbes and other antigens that mediate and regulate immune and inflammatory reactions

1.3.1 Tumor necrosis factor-alpha

Tumor necrosis factor-alpha (TNF-α) is produced during immune and host defense responses as a primary mediator of immune regulation and the inflammatory response. The major cellular source of TNF-α is activated mononuclear phagocytes, although antigen-stimulated T cells, Natural Killer (NK) cells, and mast cells can also secrete this protein The diverse role of TNF-α in mediating cellular responses are: the activation and induction of other cytokines such as IL-1, 6, 8, IFN-γ, transforming growth factor-beta (TGF-ß), in monocytes-macrophages and inhibition of differentiation and suppression of proliferation of these cells.

1.3.2. Interleukine-6

Interleukin-6 is a multifunctional cytokine, which is produced by lymphoid and non-lymphoid cells, such as fibroblasts, macrophages, dendritic cells, T and B lymphocytzes, endothelial cells, glial cells and keratinocytes. IL-6 regulates immune responses, acute-phase reactions and haematopoiesis. The production of IL-6 is regulated by a variety of stimuli, such as IL-1, TNF, IFN-β. IL-6 induces terminal differentation of B cells to antibody producing plasma cells.

1.3.3. Transforming growth factor-beta (TGF-β)

Virtually every cell in the body, including epithelial, endothelial, hematopietic, neuronal, and connective-tissue cells produces TGF-ß and has receptor for it. TGF-ß regulates the proliferation and differentiation of cells, embryonic development, wound healing, and angiogenesis. TGF-ß is one of the most potent regulators of the production and deposition of extracellular matrix. TGF-ß stimulates fibroblasts and other cells to produce extracellular-matrix proteins and cell-adhesion proteins, including collagen, fibronectin, and integrins.

Increases or decreases in the production of TGF-ß have been linked to numerous disease states, including atherosclerosis and fibrotic disease of the kidney, lung and liver.

1.3.3.1 *SNP of TGF-ß1 gene*

It has been demonstrated that the production of TGF-ß varies from individual to individual and partly depends on the polymorphisms of these genes. The human gene encoding TGF-ß1 is located on chromosome 19q13. Several SNPs have been described in the TGF-ß1 gene, including a T-to-C transition at nucleotide 29 at position +869. It has been shown that TT homozygous genotypes are high TGF-β1 producers. The correlation between the TGF-ß1 gene polymorphism and the disease status has been studied in a diverse range of diseases such as heart diseases, acute human liver graft rejection, idiopathic pulmonary fibrosis, hypertension, myocardial infarction, atherosclerosis, colon, ovarian, breast cancers; diabetic nephropathy, asthma, chronic obstructive pulmonary disease, multiple sclerosis and osteoporosis.

1.4. Surgical treatment of CP

Approximately 20-30% of patients with chronic pancreatitis (CP) develop enlargement of the head of the pancreas in consequence of inflammatory alterations, which leads to complications such as obstruction of the pancreatic duct, common bile duct stenosis and duodenal compression. All of these are indications for surgical treatment. Two types of surgical procedures are currently applied in clinical practice: drainage procedures and resection operations. Drainage procedures involve a maximal preservation of the pancreatic tissue with good early results, which decrease significantly during the follow-up examination. Resection operations comprise different types of pancreatic head resection, i.e. conventional pancreaticoduodenectomy (Whipple operation), pylorus-preserving pancreaticoduodenectomy (PPPD), Beger's duodenum-preserving pancreatic head resection (DPPHR) and Frey's longitudinal pancreaticojejeunostomy combined with local pancreatic head excision (LPJ-LPHE). In accordance with the modern organ-preserving concept, a safe procedure has been developed for duodenum-preserving pancreatic head resection in patients with CP.

Despite the successful, modern surgical intervention in a few cases we found recurrence of the disease, and late problems. We tried to get the reason helping us our results on the basis of cytokine patomechanism in pancreatitis.

2. AIMS OF THE STUDY

As cytokines have a pivotal role in the development of acute and chronic pancreatitis, the aim of our study to investigate the role of TNF-α, IL-6 and TGF-β in both diseases. In the first part of the study experimental acute pancreatitis models were applied to explore the relevance of the cytokines in the pathomechanism of acute pancreatitis. Secondly, the role of TGF-β in the chronic pancreatitis was investigated in the clinical practice involving patients with CP. Patients with CP were stratified as to patients with mild and severe form. In the case of the severe form of CP, a new organ preserving pancreatic head resection was elaborated. Description of this new method serves the third part of the thesis; the genetic predisposion of these patients is also discussed.

The present study was designated to address the following aims:

1.: To determine the changes in serum levels of TNF-α and IL-6 with time in two experimental acute pancreatitis models in rats. To find the possibility of correlation between the degree of pancreatic tissue injury in interstitial or biliary-type acute pancreatitis and serum cytokine level was studied

2.: To investigate whether changes occur in the serum TGF-β1 levels during pancreas regeneration, and whether there is a connection between their levels and the rate of the regeneration in rats.

3.: To develop a safe and effective procedure for organ-preserving pancreatic head resection, for definitive control of the complications following the inflammatory alterations of CP.

4.: To investigate whether TGF-β1 play a role in the development of CP in human, and to find a prognostic factor for the successful treatment of CP.

3. MATERIALS AND METHODS

3.1., Experimental pancreatitis

3.1.1. Acut pancreatitis modells

3.1.1.1. *Animals*

Male Wistar rats weighing 280-330 g were used in all experiments.

3.1.1.2. *CCK-8- induced acute pancreatitis*

The CCK-8-induced acute pancreatitis group (n=5) received 75 ug/kg CCK-8 (sytethysed by Botond Penke, Department of Medical Chemistry, Szeged) subcutaneously (s.c.) three times at hourly intervals. The control group of animals (n=5) received physiological saline s.c.

3.1.1.2. *PBDL-induced acute pancreatitis*

The PBDL-induced acute pancreatitis group (n=5) were anesthetized with pentobarbital and midline laparotomy was performed. PBDL was achived by ligating the common pancreato-biliary duct adjacent to the duodenal wall. The control group of animals (n=5) were subjected to a midline laparotomy (sham operation)

3.1.1.3. *Experimental protocol*

Animals were killed by abdominal aorta exsanguination 0, 2, 4, 8, 16, 24 or 48h following the last CCK-8 injection or PBDL. The pancreas was carefully removed, cleaned of fat and weighed.

3.1.1.4. *Histological examination*

A fragment of the pancreas was fixed overnight in 10% neutral formaldehyde solution for hematoxylin and eosin stuning and for histological study by light microscopy.

3.1.2. Pancreas regeneration models

3.1.2.1. *Animals*

Male Wistar rats weighing 350-450 g were used in all experiments.

3.1.2.2. *CCK-8-induced regeneration*

In each group the rats (n=5) were anesthetized with ether and midline laparotomy was performed. The distal part (75%) of the pancreas was resecated with preserving the spleen. CCK-8 was administered subcutaneously in a 300 ng/kg dose 3 times per day to the investigated group, while the control animals received the same amount of saline.

3.1.2.3. *Experimental protocol*

Animals were killed by abdominal aorta exsanguination 3, 7, 14, and 28 days after the first injection. The residual pancreas was carefully removed, cleaned of fat and weighed. The changes in the pancreas weight were calculated in each case to the formerly resecated weights respectively, by mathematical methods.

3.1.3 Assays

3.1.3.1. *Amylase activity*

Serum amylase activity was measured by the Phadebas test method.

3.1.3.2. *TNF and IL-6 assay*

TNF and IL-6 bioassay was performed applying WEHI 164 targets.

3.1.3.3. *Western blot analysis for TGF-β*

TGF-β was detected by an ECL-Western blot technique with the application of anti-TGF-β1 antibody.

3.1.3.4. *DNA and protein content*

DNA contents of the pancreas were determined by the procedure of Giles&Meyers, protein content by the GOA method; assayed with colorimetric method.

3.1.3.5. *TGF-β assay*

For TGF-β ELISA determination venous blood was collected from the rats into EDTA-containing tubes for collecting plasma. Blood was collected 3, 7, 14, and 28 days after the first CCK-8 injection. Centrifugation was carried out at 2000g for 10 min at 4^{o}C. All samples were stored at -20 ^{o}C. Plasma concentration of TGF-β1 was determined by enzyme-linked immunosorbent assay kit (R&D System Inc., Minneapolis, USA) according to the instructions of the manufacturer.

3.1.4. Statistical analysis

Results were expressed as mean ± SEM. Experiments were evaluated statistically with Student's t-test for paired or unpaired values, as appropriate. *P* values less than 0.05 were accepted as significant.

3.2., Human chronic pancreatitis

3.2.1.: The role of TGF-β1 in patients with CP

3.2.1.1. *Patients*

Our study involved 83 patients (24 females and 59 males; mean age 52.7 years, range 22-70) who underwent medical or surgical treatment for chronic pancreatitis at the Department of Internal Medicine and/or Department of Surgery of the University of Szeged between 2003 and 2006. The diagnosis of chronic pancreatitis was based on the typical history (daily alcohol intake), abdominal complaints (pain, bloating, steatorrhoea, etc.) and characteristic morphologic and/or functional alterations of the pancreas. The morphologic changes due to chronic inflammation of the pancreas (pancreatic calcification on ultrasonography /US/ and/or computed tomography /CT/, mild to moderate or marked ductal lesions during endoscopic retrograde cholangio-pancreatography- /ERCP/ examination) were assessed in each case. Pancreatic calcifications were found in 31 (37.5 %) patients on US or CT.

3.2.1.2. *Surgical intervention*

The indication for operation was intractable pain, loss of body weight, and obstruction of the ductal system (pancreatic duct, common bile duct or the duodenum) caused by an inflammatory enlargement of the pancreatic head. Duodenum preserving pancreatic head resection was performed in 8 cases, organ preserving pancreatic head resection in 15 cases, pylorus preserving pancreatic head resection in 4 cases, and Wirsungo-jejunostomy in 13 cases. Rehospitalization and reoperation was necessary in 8 cases.The control group consisted

of 75 age- and gender-matched healthy blood donors, who had no gastrointestinal or liver diseases, were selected locally from consecutive blood donors in Szeged, Hungary.

3.2.1.3. *DNA extraction*

For the examination of TGF-ß1 polymorphisms, genomic DNA purified from peripheral blood was used.

3.2.1.4. *Determination of TGF-ß1 +869 T→C polymorphism*

The defined single-nucleotide polymorphism T^{29}-C in exon 1 of the human TGF-ß1 gene was determined with an amplification refractory mutation system –ARMS- with a generic primer (sense), (5'-TCCGTGGGATACTGAGACACC-3'); and with two allele-specific antisense primers, differing from each other in only one base at the 3'-end- primer C: 5'-GCAGCGGTAGCAGCAGCG-3' and primer T: 5'-AGCAGCGGTAGCAGCAGCA-3'

3.2.1.5. *TGF-β1 ELISA*

Venous blood was collected from healthy blood donors and patients with chronic pancreatitis into EDTA-containing tubes for collecting plasma.

3.2.1.6. *Statistical analysis*

Statistical analyses for comparison of allele and genotype frequencies between groups were performed by using the χ^2 test and Fisher's exact test if one cell had $n < 5$.

3.2.2.: New organ preserving pancreatic head resection in patients with CP

3.2.2.1. *Patients*

Since February 1999, a new surgical procedure has been performed in 135 patients (103 men and 32 women; mean age: 49.5 yrs [range 28-63]) after the development of an inflammatory tumor of the pancreatic head (median diameter 68 mm [range 46 to 129 mm], as assessed by helical CT scan). The etiology was connected with chronic alcohol ingestion in 86% (117 patients), the CP was associated with biliary stone disease in 14 patients (10%), and it was unknown in 4 patients. The diagnosis was confirmed by ERCP, sonography and the CT scan.

3.2.2.2. *Operative procedure*

The operative procedure started with the Kocher maneuver, partial dissection of the gastrocolic ligament for mobilization, and exploration of the head of the pancreas, without division and cutting of the pancreas over the portal vein. An intraoperative frozen section was performed for all patients; none of them revealed signs of malignancy. The following step of the operative procedure was ligation of the pancreaticoduodenal artery and the veins directed to the duodenum and to the superior mesenteric vein. The enlarged pancreatic head was excised in almost its entirety, leaving behind a bridge of pancreatic tissue about 10 mm wide, while a rim of pancreas (5 to 10 mm) remained beside the duodenum and on the upper margin

of the pancreatic head. This wide excision gives a possibility for drainage of the pancreatic juice from the distal pancreas and for opening of the prepapillary obstructed common bile duct in the icteric patients and in patients with a stenotic common bile duct. The prestenotic dilatated common bile duct was opened with an incision about 8-10 mm long, and the opened duct wall was sutured to the surrounding pancreatic tissue with interrupted Vicryl® 3/0 sutures. After careful hemostasis of the operative region, the reconstruction, with drainage of the secretion from the remaining pancreas into the intestinal tract, took place through a jejunal Roux-en-Y loop, with application of one-layer interrupted Vicryl® 2/0 sutures. There was no indication or necessity for blood transfusion during the operation. The mean operating time was 165 min (range 120 to 210 min).

3.2.2.3. *Quality of life*

The QoL and pain score before and after surgeries were assessed and pain intensity was estimated.

3.2.2.4. *Statistical analysis*

Statistical significance was estimated by using Student's t test or the Wilcoxon rank test, as appropriate. The level of significance was set at $P<0.05$.

4. RESULTS

4.1. Experimental acute pancreatitis

4.1.1: CCK-8-induced pancreatitis

The ratio pw/bw was significantly increased after the induction of acute pancreatitis and reached its maximum level 4 h after the last CCK-8 injection (8.19±1.13 vs. 4.72±0.64 mg/g). After 4 h, pw/bw decreased continuously, and it had normalized by 24 h. The serum amylase activity rose steadily up to 4 h after the last CCK-8 injection ($69.4\pm12.8\times10^3$ vs.$3.12\pm0.28\times10^3$ U/ml). It had reached the control level at 16 h.

In the CCK-8-induced pancreatitis, the serum IL-6 level had begun to increase at 2 h, but the peak level (123.3±5.7 pg/ml) was reached at 4 h. Thereafter it decreased, but it was stil' higher than the control (37.5±15 pg/ml). Detectable TNF was found in CCK-8-induced pancreatitis only at 2 h (25.5±5 U/ml).

Macroscopic study of the pancreata in the CCK-8-treated gropup revealed interstitial edema. Microscopic examination demonstrated a moderate diffuse parenchymal degeneration, including vacuolation and focal necrosis (dark cells) within the acinar cells. These

morphological changes were most evident at 8h and gradually decreased thereafter. The interstitium remained virtually unchanged.

4.1.2. PBDL-induced acute pancreatitis

In PBDL-induced pancreatitis, the increase in pw/bw was fairly contiuous and it reached its maximum leve lat 48 h (8.8±1.4 vs. 5.3±0.8 mg/g). The serum amylase acuvity peaked 2 h after PBDL (43.2±13x10^3 U/ml), and then decreased continuously. The maximum elevation in serum IL-6 level int he PBDL model was observed at 16 h (3800±447 pg/ml). The laparotomy itself (sham operation) also resulted in a moderate elevation of the serum IL-6.

On visual inspection, the pancreata were observed to be enlarged and edematous, with occasional hemorrhages. Histologically, increasing edema and infiltration by neutrophilic leukocytes and mononuclear phogocytes were seen int he interstitium of the pancreas 2 h after ligation. The acinar lumen was dilated and filled with secretion, and the acinar cells were partly degranulated. In contrast, the ducts and blood vessels remained unaltered. These changes progressed during the experiment. By 8 and 16 h after the ligation, cytoplasmic vascuolation of the acinar cells was seen and the acinar cells apeeared to be necrotic. By 24 and 48 h, areas of intraparenchymal hemorrhage were observed.

4.1.3. CCK-8 induced pancreas regeneration

In the *pancreas regeneration model the wet weights* of the residual pancreas increased in both groups up to day 3. Subsequently, the weights decreased in the controls, but increased continuously in the CCK-8-treated group. There was a significant difference on day 14, and on day 28 the pancreas weight was almost doubled in the CCK-8-group, whereas in the controls it has decreased to normal level.

The protein content reached its highest level on day 28 in the CCK-8-treated group (32.435±7.88 mg/pancreas). A significantly higher level of IL-6 was measured on days 7 vs. the control (250±70 v. 50±30 pg/ml). It later decreased, but remained above the control level.

The DNA content of the pancreas was continuously higher in the treated than in the control group. It reached the maximum level on day 28, with significant difference (1850±350 vs. 780±240 γ/pancreas).

Significantly different *TGF-β1 levels* were measured on days 7 and 14 (290±40 vs. 155±60 and 295±8 vs. 155±55 ng/ml, respectively). There was no difference between the TGF-B1 levels in the two groups on day 28.

Western blotting was applied for the immunodetection of TGF-β in protein lysates of pancreas samples, following CCK induction. TGF-β expression in the rat pancreas increased from.3 days following CCK treatment up to the 7th day, thereafter the TGF-β level returned

to the basal level.,These data underline the role of TGF-β in the regeneration in experimental pancreatitis.

No significant changes were observed in the *amylase levels*; they remained at a normal level (5.3±0.5 U/ml). This indicates that he increase in the pancreas weight was not caused by pancreatitis.

4.2.: Human chronic pancreatitis

4.2.1. Experimental results

4.2.1.1. *TGF-β1 +869 T→C polymorphism in patients with CP*

There was a significant difference in genotypic distribution between the chronic pancreatic patients overall and the healthy controls ($p = 0.009$, $\chi^2 = 9.409$). The frequency of TT homozygote's (high TGF-β1-producing phenotype) were significantly higher in the patient group overall (50%) than in the controls (28%) (p=0.005; OR =2.634; 95% CI = 1.358-5.111). There was an even higher frequency of the TT genotype among patients with surgical intervention as compared with the controls , 62 % vs. 28 %, p = 0.0007, OR = 4.018, 95 % CI = 1.796 - 8.987 There was also a significant difference between the operated patients, and those treated medically (p = 0.0486, OR=2.549, 95%CI=1.052-6.178). Though the frequency of the TT genotype was still higher among the patients in the medically treated (non - operated) group (39.5%) than in the controls, the difference was not statistically significant. No further significant differences were observed as regards the SNP-s when the patients were stratified according to the presence or absence of calcification. The frequency of the T/C genotype was significantly higher in both groups of patients than in the controls (58 %, and 58% vs. 40%)

4.2.1.2. *TGF-β1 plasma levels*

Plasma levels of TGF-β1 were higher in the patients overall than in controls (3.98 ±1.26 ng/ml vs. 2.1 ± 0.85 ng/ml), and higher in the patients with TT genotype than in those with the CT and the CC genotypes (5.2 ± 1.7 ng/ml vs. 3.8 ± 1.1 ng/ml and vs. 1.5 ± 0.5ng/ml respectively; p< 0.001 ANOVA). A similar tendency was observed in the control group; the subjects with TT genotype exerted the highest plasma TGF-β1 levels (2.8 ± 0.9 ng/ml). However, the plasma TGF-β1 concentrations differed significantly between the patients and the controls, both in the TT homozygote groups and in the TC heterozygote groups. (p < 0.001 statistically significant are according the Bonferroni post test.) No significant difference was observed between the"low - level" TGF-β1 concentrations when the patients and controls

were CC homozygote. Those patients who had TT genotype with high serum levels of TGF-β1 were rehospitalized or underwent further operations.

4.2.2. Surgical results

In 135 patients, the OPPHR procedure was performed after the development of an inflammatory tumour of the pancreatic head. The mean follow-up period was 4.1 years (range 0.5 to 7.0). Five patients were lost to follow-up (3.7%). 116 patients became complaint-free (89%), 14 patients had moderate symptoms and the body weight increased by a mean of 11.3 kg (range 4-28) ($P<0.05$). Within 2 years following operations, 5 patients were reoperated: a bilio-digestive bypass was performed in consequence of developed bile duct stenosis. In the follow-up period, a further 6 patients were admitted to the clinic with an acute episode of pancreatitis; all of them were treated conservatively. Readmission was therefore necessary 11 of the 130 patients (8.4%). The late mortality was 3.7% (5 patients); the reason was cardiovascular failure or an accident, 4 and 1 patient respectively. Both before the operation and during the follow-up, the patients were asked to complete the QoL questionnaire. The median pain score decreased by 91% ($P<0.001$) after surgery. No patient suffered a frequent pain attack and only 10% of the patients mentioned moderate pain occasionally without any pain killer medication.

5. DISCUSSION

5.1., Both the administration of high dose of CCK-8 and ligation of the pancreato-biliary duct are suitable methods for the induction of acute pancreatitis, but biochemical and morphological examination revealed differences in the severity of changes. The over dosage of CCK-8 resulted in a mild oedematous (interstitial) pancreatitis, while PBDL caused a more severe necro-hemorrhagic pancreatitis .

The serum IL-6 level began to rise after the induction of acute pancreatitis and its peak preceded the most severe morphological alterations and the maximum amylase concentration in both models. The maximum IL-6 level was 30 times higher in the PBDL model than in the interstitial pancreatitis model. It is noteworthy that the sham operation itself caused a transient elevation of the serum IL-6 level. In human, an increased serum IL-6 concentration has been observed after abdominal surgery. This might be the explanation for the increased cytokine levels in the sham-operated control animals.

Only a moderate TNF-α elevation was detected in CCK-8 induced acute pancreatitis, whereas its level was increased about 10-fold in the PBDL group. In the PBDL model particularly high

IL-6 levels were observed and, concomitantly, significantly higher TNF concentrations could be detected. We could demonstrate correlations between the laboratory and morphological parameters of pancreatitis and the serum IL-6 levels in both experimental acute pancreatitis models.

5.2., Our study revealed a transient increase in TGF-β1 levels in the CCK-8 treated group. The significant changes in the pancreas weight and the protein and DNA contents indicated pancreas regeneration and some degree of fibrosis. The TGF-β1 level remained high until day 14, and then decreased. Additionally the pancreas tropism induced by CCK-8 could be detected in increasing level up to the end of the first month. After cerulein-induced pancreatitis, TGF-β1 was found to be involved in the regulation of extra cellular matrix regeneration. These data show that cytokines can take part and modulate the development of the pancreas, and suggest roles for TGF-β1 in regulating the in vivo regeneration of the pancreas.

5.3., In approximately 30% of patients with CP, the disease is primarily located in the head of the pancreas, which is known to act as the "pacemaker" to trigger the inflammatory process; resection of this inflammatory mass must be regarded as pivotal in the surgical intervention.

Our data on 135 operated patients demonstrated that OPPHR is a safe operative procedure, as confirmed by the low morbidity (2.9%) and the absence of mortality among the patients in the postoperative period. An additional important feature is that the median duration of hospitalization was only 8.5 days. In the mean follow-up period of 4.1 years (range 0.5 to 7.0), 116 patients became complaint-free (89%), while 14 had moderate symptoms and the body weight increased significantly by a mean of 11.3 kg (range 4-28). Readmission was required for 11 of the 130 patients (8.4%) as a consequence of relaparotomy (bilio-digestive bypass) or conservatively treated pancreatitis. The late mortality was 3.7%: 5 patients died. The preoperative and postoperative endocrine function remained in almost the same stage.

During the follow-up, the median global QoL improved by 100%. Apart from the cognitive functioning, the physical status, working ability, and emotional and social functioning all improved significantly ($P<0.05$).

5.4., No correlation was found between the genotypes and the histological varieties of chronic pancreatitis. The differences in TT genotype frequency proved significant between the operated group and the controls, and between the operated and the non operated group. The frequency of the TT genotype was relatively high among the medically treated patients, but only as a tendency without statistical significance.

It is noteworthy, that reoperation was necessary within 3 years in 8 patients; all of them were TT homozygote. The highest TGF-β concentrations (5.2 - 7.4 ng/ml) were detected in the plasma of these patients.

Higher concentrations of TGF-β1 were detected in the plasma of the subjects with the TT and TC genotypes as compared with those with the CC genotype both among the patients and among the controls. The frequency of high producers (TT) was higher among the patients with chronic pancreatitis than among the controls, and the TGF-β1 levels differed in the patient and control groups. The frequency of the T/C genotype was significantly higher in both groups of patients than in the controls (58 %, and 58% vs. 40%). All the successfully treated patients carried the genotypes of T/C or C/C with low serum levels of TGF-β1.

In conclusion, it is very likely that the TGF-β1 polymorphisms contribute to the genetic susceptibility to chronic pancreatitis. The TGF-β1 genetic polymorphism with higher TGF-β1 production appeared relevant among the patients with chronic pancreatitis, who underwent surgery; particularly, when reoperations were necessary. The prognostic value of TGF - β1 polymorphism and the associated TGF-β1 levels should be determined in a future follow-up study on a larger series of patients.

From this point of view, if we would like to answer whether *Can we treat patients with chronic pancreatitis successfully?* - The answer is: hopefully YES (if we are prepared to consider also the immunological risk factors…)

6. ACKNOWLEDGEMENTS

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List of full papers cited in the Thesis

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